Promotor screening with reporter proteins for the yeast *Starmerella bombicola*

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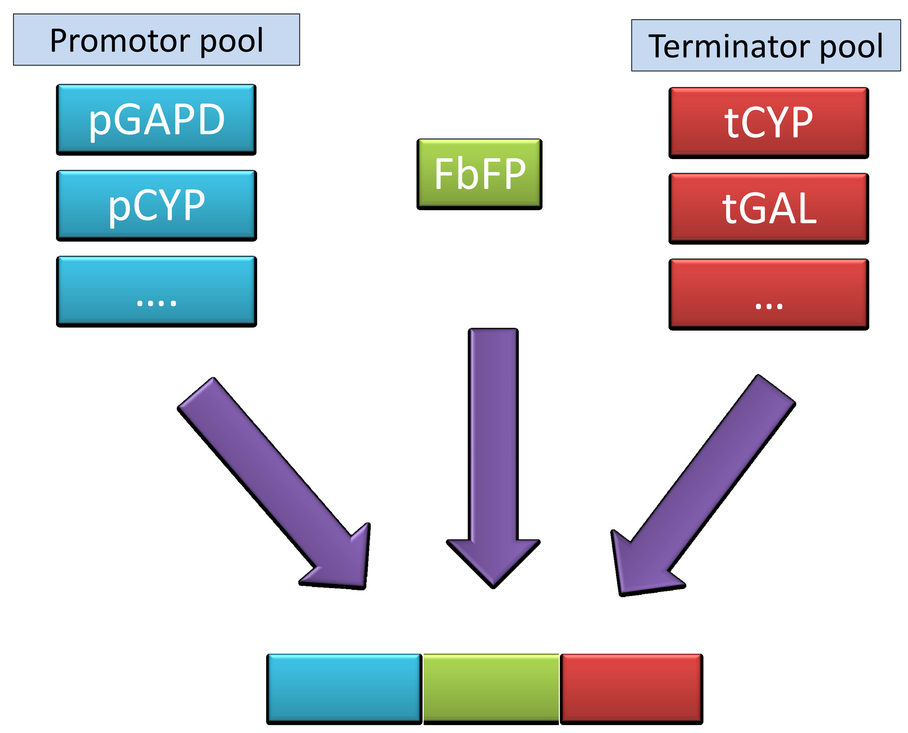
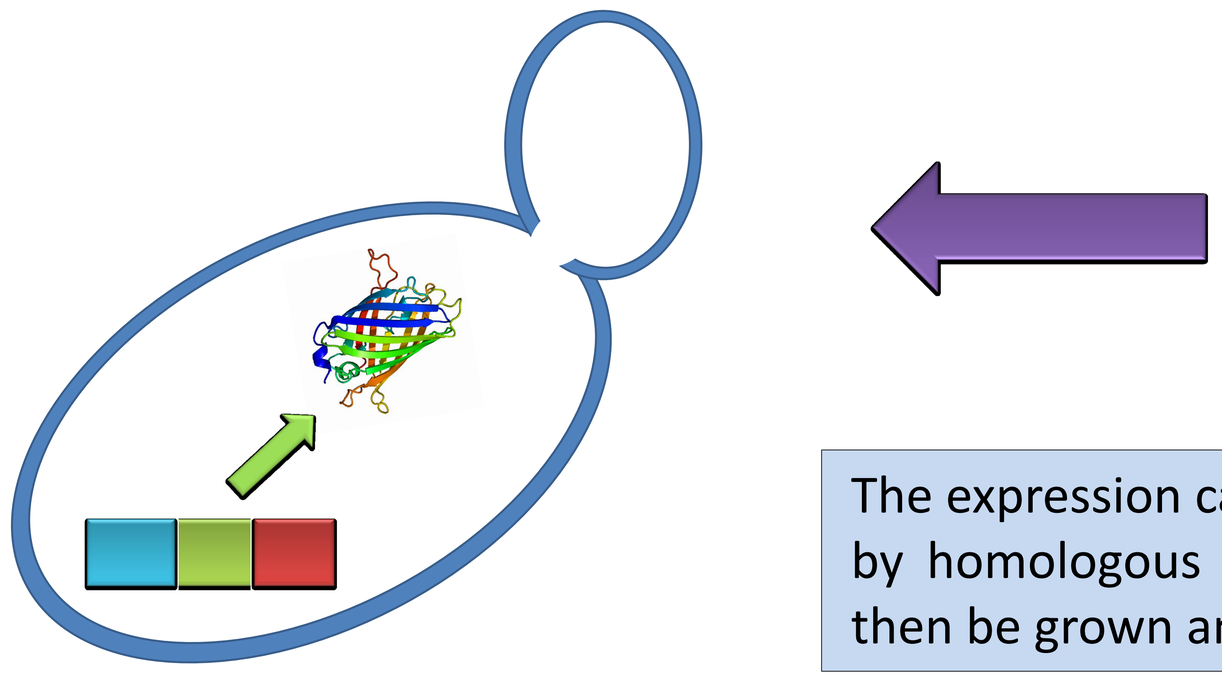
Introduction

During the years, the yeast *Starmerella bombicola* has proven itself as an interesting organism. The wild-type strain produces for example sophorolipids, a kind of glycolipid biosurfactant, at high concentrations. Engineering of the sophorolipid biosynthesis pathway, either by overexpressing or knocking-out certain genes, resulted in industrially interesting strains. Not only sophorolipids, but also cellobiose lipids and polyhydroxyalkanoates have been succesfully synthesized. The aim is to turn this yeast into a platform organism for the production of other interesting biomolecules. For this, deeper molecular knowledge is required. Several codon optimised reporter proteins, Green Fluorescent Protein and Amylase, have already been used to gain novel insights. By using a combinatorial approach various promotors and terminators were combined in different expression cassettes for several interesting loci so that both intra- and extracellular measurements could be done. A new reporter protein, flavin mononucleotide (FMN)-binding fluorescent protein (FbFP)[1], will be tested during this research.

Setup

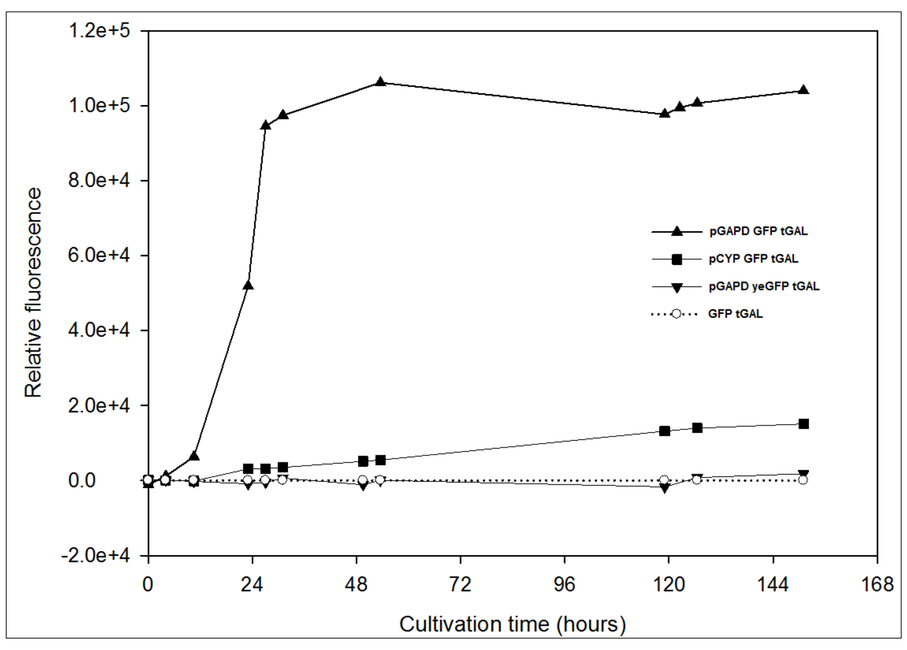
A great benefit of FbFP is that it doesn't need oxygen to work. This way, it is possible to circumvent a potential bias in reporting when insufficient oxygen is available. Several constructs with different promotors and terminators will be constructed.



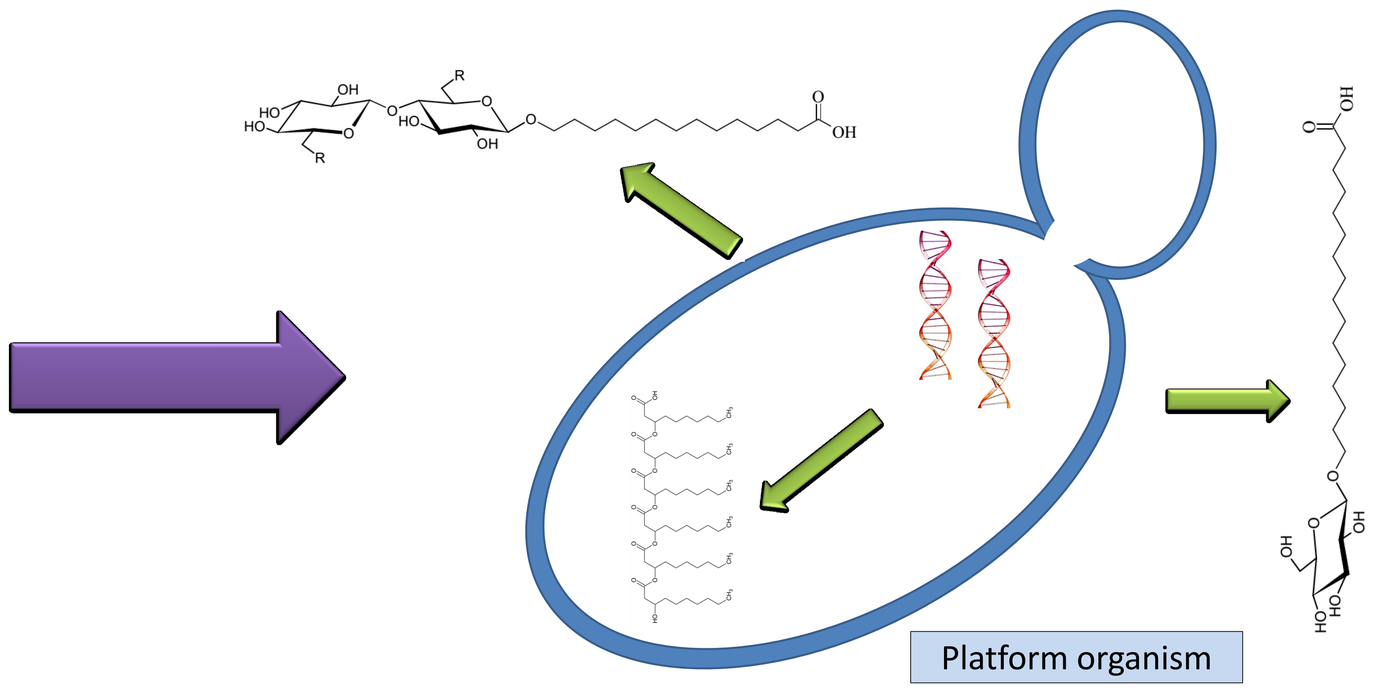


The expression cassettes will be introduced in *S. bombicola* by homologous recombination. The obtained strains will then be grown and sampled in various growth conditions.

The final result of this will be the creation of a promotor library which can be used in the future when new pathways are going to be introduced or already existing ones must be optimized. Ideally, all kinds of promotors will be accounted for. This way, more efficient metabolic engineering can be obtained.



Results obtained for constructs at the ura3-locus with the *cyp52m1* and *gapd* promotors and codon optimised GFP on the sophorolipid production medium[2].



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1. Drepper, T., Huber, R, Heck, A., Circolone, F., Hillmer, A-K., Jochen Büchs, J. & Jaeger K-E.; 2010
2. Roelants, S.; 2013; Doctoral thesis